



ISSN (E): 2277- 7695

ISSN (P): 2349-8242

NAAS Rating: 5.23

TPI 2021; SP-10(4): 135-138

© 2021 TPI

www.thepharmajournal.com

Received: 10-02-2021

Accepted: 12-03-2021

Pankaj Kumar

Department of Veterinary Parasitology,
College of Veterinary Science & Animal Husbandry, Odisha University of Agriculture & Technology, Bhubaneswar, Odisha, India

Bijayendranath Mohanty

Department of Veterinary Parasitology,
College of Veterinary Science & Animal Husbandry, Odisha University of Agriculture & Technology, Bhubaneswar, Odisha, India

Dr. Manaswini Dehuri

Assistant Professor, Department of Veterinary Parasitology, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar, Orissa, India

Sushant Kumar Dash

Department of Animal Breeding & Genetics, College of Veterinary Science & Animal Husbandry, Odisha University of Agriculture & Technology, Bhubaneswar, Odisha, India

Susen Kumar Panda

Department of Veterinary Pathology, College of Veterinary Science & Animal Husbandry, Odisha University of Agriculture & Technology, Bhubaneswar, Odisha, India

Prakash Chandra Behera

Department of Veterinary Biochemistry, College of Veterinary Science & Animal Husbandry, Odisha University of Agriculture & Technology, Bhubaneswar, Odisha, India

Akshaya Kumar Kundu

Department of Veterinary Physiology, College of Veterinary Science & Animal Husbandry, Odisha University of Agriculture & Technology, Bhubaneswar, Odisha, India

Ananta Hembram

Department of Veterinary Parasitology, College of Veterinary Science & Animal Husbandry, Odisha University of Agriculture & Technology, Bhubaneswar, Odisha, India

Corresponding Author:

Dr. Manaswini Dehuri

Assistant Professor, Department of Veterinary Parasitology, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar, Orissa, India

Detection of benzimidazole resistance against naturally occurring gastrointestinal nematodes in different sheep breeds of Odisha

Pankaj Kumar, Bijayendranath Mohanty, Dr. Manaswini Dehuri, Sushant Kumar Dash, Susen Kumar Panda, Prakash Chandra Behera, Akshaya Kumar Kundu and Ananta Hembram

DOI: <https://doi.org/10.22271/tpi.2021.v10.i4Sc.5994>

Abstract

Gastrointestinal nematodes are a major hindrance in sheep husbandry and the efficient management of these parasites is curbed by the development of anthelmintic resistance. The present investigation was carried in different sheep breeds of Odisha and the status of resistance in gastrointestinal nematodes against Fenbendazole was carried out by *in vivo* faecal egg count reduction test (FECRT) and *in vitro* Egg Hatch Assay (EHA) test. The results of the anthelmintic resistance study indicated benzimidazole resistance to gastrointestinal nematodes in *Kendrapada*, *Ganjam* and *Nondescript* breed of sheep while *Balangir* breed of sheep where found susceptible.

Keywords: fenbendazole, gastrointestinal nematodes, sheep, anthelmintic resistance

Introduction

Sheep rearing is one of the oldest occupations adopted by livestock farmers in India as it is a source for providing protein nutrition to the family and also for earning additional income. In, Odisha, there are three descriptive breeds of sheep native to state along with nondescript breeds. The recognized breeds of sheep in Odisha are, *Balangir*, *Ganjam*, and *Kendrapada*. Parasitism is an important global problem and still continues to seriously affect the livestock economy throughout the world. Parasitic gastroenteritis is caused by the gastrointestinal (GI) nematodes of ruminants and is characterized by diarrhoea, anorexia, lethargy, anaemia, sub-mandibular oedema and death particularly in young animals in severe cases (Sargison *et al.* 2002, Taylor *et al.* 2007) [19]. The use of anthelmintic drugs has been the common and most reliable practice since decades for effective control of gastro-intestinal nematodes in grazing animals including sheep. Since 1960's benzimidazoles (BZ) are the most extensively used anthelmintics which were found most effective (>95%) for control of Strongyle nematode infection in sheep and goats (Dorny, *et al.*, 1995). Benzimidazoles get preference for use due to their low cost, broad spectrum activities and high efficacy. But their indiscriminate use over years has led to the development of resistance in the parasites (Waller, 1994) [20]. The first report of occurrence of resistance to BZs (Thiabendazole) in *H. contortus* was in 1961 and as the time passed this problem became widespread across globe (Wolstenholme *et al.*, 2004) [21]. For detection of BZ resistance, several techniques have been practiced i.e., *in vivo* and *in vitro* assays. The faecal egg count reduction test (FECRT), egg hatch assay (EHA) and larval development assay (LDA) have proven to be suitable tests for detecting BZ resistance (Coles *et al.* 2006) [2]. Studies on the status of Benzimidazole resistance in sheep has been reported by many researcher in different states of the country (Das and Singh, 2005; Rialch *et al.* 2013; Singh *et al.* 2015; Lata 2018) [3, 16, 13]. Report on detection of anthelmintic resistance in Odisha is meager (Sahu 2015, Nanda 2016) [14]. Barring these few reports no systematic investigation on the status of anthelmintic resistance among the prevalent population of *Haemonchus contortus* and other GI nematodes of sheep in Odisha in the native breeds of sheep to *Haemonchus contortus* has been undertaken till date. The present research was undertaken keeping in view the above facts.

Materials and Methods

The *in vivo* evaluation of anthelmintic resistance under field condition was done by Faecal egg count reduction test (FECRT) following the standard guidelines of W.A.A.V.P. Sheep belonging to different breeds in their respective native tracts which were detected naturally infected with GI nematodes were included in the study. Flocks which were not dewormed during last three months and found positive for GI nematode eggs with a minimum Egg per gram (EPG) of 150 and above were finally selected, marked with identification number on their fleece and divided into treated and control groups. Ten animals in each group were selected from the *Kendrapada*, *Ganjam*, *Balangir* and non-descript, sheep breeds. The EPG Pretreatment was done before administration of anthelmintic (Fenbendazole @ 7.5 mg/kg body weight orally as a single dose) and EPG post treatment 10 days after drug administration. Per-rectally collected dung samples from sheep were kept separately in plastic containers with screw cap in an anaerobic condition without adding any preservative. The EPG count of both the treated and control groups were done by Mc. Master's technique (Soulsby, 1982) [17].

The faecal egg count reduction percentages of sheep naturally infected with gastrointestinal nematodes were calculated by comparing the pre treatment and post treatment EPG.

$$\text{FECE \%} = \frac{\{(\text{EPG pre treatment} - \text{EPG post treatment})\}}{\text{EPG pre treatment}} \times 100$$

A reduction in faecal egg count less than 95% as well as lower 95% confidence level below 90 was taken as criteria to indicate the presence of benzimidazole anthelmintic resistant nematodes in the treated population (Coles *et al.*, 1992) [1]. On 11th day the control group animals were also treated with the same drug to make them free from natural infection.

Egg Hatch Test (EHT)

Collection of Eggs

Freshly collected and anaerobic stored dung samples were homogenized using pestle and mortar. The homogenized dung sample was filtered, filtrate was centrifuged at 2000 rpm for 2 minutes and the sediment was cleaned with distilled water by centrifugation. Then saturated salt (NaCl) solution was mixed to sediment and centrifuged. The supernatant solution containing eggs was collected in centrifuge tube and washed in distilled water for three times by centrifugation. Finally, the supernatant was discarded and the sediment was resuspended with distilled water. Finally, the number of eggs was estimated and diluted to 100-150 eggs/ml.

Preparation of Thiabendazole (TBZ) stock solution

A stock solution was prepared as described by Himmelstjerna *et al.* (2009) [5]. TBZ 50 mg powder was dissolved in 5 ml of dimethylsulphoxide (DMSO) in a test tube (Solution A). Then 1 ml from Solution A was added to 9 ml of DMSO (Solution B) having a concentration of 1 mg TBZ per ml. Different concentration of working solution were prepared.

The working dilution were made for 24 well culture plate. In each well, 1 ml of egg suspension were added to 10 µl of thiabendazole in different concentrations and mixed well. In control well, 10 µl of dimethylsulphoxide (DMSO) was added with 1 ml of egg suspension. The 24-well culture plate were placed in the incubator for 48 hours at 26°C. The incubation was terminated by adding 2- drops of Lugol's Iodine to each well and embryonated eggs, unhatched eggs and hatched first stage larva from each well were counted and proportion of egg hatch was determined. The trial was conducted with two replicates and result was expressed as ED₅₀ values.

$$\text{EHA \%} = \frac{\text{No. of hatched larvae counted}}{\text{Total no of eggs and larvae}} \times 100$$

Effective Dose₅₀ (ED₅₀) value was calculated for the eggs by log probit analysis. Eggs having ED₅₀ value exceeding 0.1 µg BZ anthelmintic per ml was indicative of resistance against Benzimidazole (Coles *et al.* 2006) [2].

Statistical Analysis

A log probit model was used to estimate the ED₅₀ of the concentration of the drug based on the hatching percentage in EHA by using SPSS 21.

Results and Discussion

The efficacy of fenbendazole was assessed by faecal egg count reduction test (FECRT) in different sheep breeds of Odisha naturally infected with GI nematode. Faecal egg count reduction was recorded based on pretreatment egg per gram (0-day) and post treatment egg per gram (on 10th day of oral administration of Fenbendazole @ 7.5mg/kg.) and compared with the untreated control group. The efficacy of Fenbendazole in *Kendrapada*, *Ganjam*, *Balangir* and Nondescript breeds of sheep were found to be 93.55%, 91.08%, 97.10% and 90.98 % respectively (Table 1). The results reflected the resistance to gastrointestinal nematodes in *Kendrapada*, *Ganjam* and Nondescript breed of sheep, where efficacy were recorded lower (<90%) 95% confidence interval. Gastrointestinal nematodes in *Balangir* breed of sheep where recorded (>92%) 95% confidence interval was found susceptible.

Table 1: Efficacy of Benzimidazole (Fenbendazole) against naturally occurring gastrointestinal nematodes in different breeds of sheep

Breed	Pre-treatment EPG	Post-treatment EPG	FECE %	Confidence interval		Remarks
				lower	Higher	
Kendrapada	475 ± 71.59	30 ± 15.28	93.55%	87	-	Resistant
Ganjam	785 ± 52.73	70 ± 26.03	91.08%	82	99	Resistant
Non descript	610 ± 65.74	55 ± 26.30	90.98%	86	-	Resistant
Balangir	690 ± 70.91	20 ± 9.51	97.10%	92	99	Susceptible

The percentage of gastrointestinal nematode larval composition in pre and post treatment faecal samples (Table 2) reflected the dominance of *Haemonchus* sp over *Trichostrongylus* sp, *Oesophagostomum* sp and *Strongyloides* sp in *Kendrapada*, *Ganjam* and Nondescript breed of sheep. While in Balangir sheep, post treated faecal culture showed

absence of larvae. However, post treatment faecal culture revealed absence of *Strongyloides* sp. in all sheep breeds. The above pattern of survived larvae and predominance of population of *Haemonchus contortus* among them revealed that there were existence of population of *H. contortus* which were resistant to Fenbendazole and thus to BZ.

Table 2: Gastrointestinal larval population (%) in pre and post treatment faecal culture of different sheep breeds of Odisha

Breeds	Nematode larva	Pre treatment (%)	Post treatment (%)	Control (%)
Kendrapada	<i>Haemonchus</i> sp	53	75.5	54
	<i>Oesophagostomum</i> sp	19	9	14.5
	<i>Trichostrongylus</i> sp	21	15.5	22
	<i>Strongyloides</i> sp	7	0	9.5
Ganjam	<i>Haemonchus</i> sp	55.67	81.0	56
	<i>Oesophagostomum</i> sp	15	8.0	17
	<i>Trichostrongylus</i> sp	22.67	11.0	21
	<i>Strongyloides</i> sp	6.67	0	6
Balangir	<i>Haemonchus</i> sp	60	0	63.67
	<i>Oesophagostomum</i> sp	15.0	0	11.67
	<i>Trichostrongylus</i> sp	17.33	0	15.33
	<i>Strongyloides</i> sp	7.67	0	9.33
Non-descript	<i>Haemonchus</i> sp	65.30	85	64.71
	<i>Oesophagostomum</i> sp	13.86	5.0	10.57
	<i>Trichostrongylus</i> sp	15.43	10	18.4
	<i>Strongyloides</i> sp	5.41	0	6.32

Different in-vivo techniques have been employed for detection of anthelmintic resistance of which most widely used technique recommended by the WAAVP is Faecal Egg Count Reduction Test (FECRT). It is the most suitable method for field level diagnosis of anthelmintic resistance. Quantitative reduction in the faecal egg output post treatment in comparison to pre-treatment is the basis of detection of resistance. A population of worms is declared to be resistant if the percentage reduction is less than 95 % and lower limit of 95% confidence interval is below 90. If one of the two criteria is met, resistance is suspected (Coles *et al.*, 1992) [1]. But FECRT has some limitations due to lack of its analytical sensitivity. It was demonstrated that the FECRT can detect BZ-resistance if the frequency of the resistance alleles is greater than 25% in the parasitic population under test. Therefore, FECRT is not a sensitive test to detect early emergence of anthelmintic resistance (Martin *et al.*, 1989; Levecke *et al.* 2009) [10, 9].

Egg Hatch Assay (EHT) was conducted using the method recommended by WAAVP and ED₅₀ value (concentration of drug required to kill 50% of eggs) was calculated basing on egg hatch percentage. The results of EHT carried out with respect to different sheep breeds have been tabulated in Table 3. As per the recorded results of regression, Anthelmintic Resistance were found in the *Kendrapada*, *Ganjam* and Nondescript breed of sheep with 0.117102µg/ml, 0.168242µg/ml, and 0.232582µg/ml, ED₅₀ value of Thiabendazole while Balangir sheep showed no evidence of anthelmintic resistance as the regression values recorded 0.041137µg/ml (the ED₅₀ values were found less than 0.1 µg/ml).

Table 3: Egg hatch assay (EHA) against naturally occurring gastrointestinal nematodes in different breeds of sheep of Odisha

Breed	ED ₅₀ value	Remarks
Kendrapada	0.117102µg/ml	Resistant
Ganjam	0.168242µg/ml	Resistant
Nondescript	0.232582µg/ml	Resistant
Balangir	0.041137µg/ml	Susceptible

In-vitro assays such as Egg Hatch Test (EHT) are also conducted for diagnosis of anthelmintic resistance. It is advantageous because of its low cost, and having no inter-host variation, since no use of animal is required. The methodology involve the assessment of the inhibition of hatchability. In EHT the proportion of eggs that fail to hatch

in increasing drug concentrations is determined. Therefore, this test is only suitable for detection of BZ resistance (Swarnakar and Singh, 2017) [18].

The detection of benzimidazole resistance by FECRT and EHT has been previously reported from three sheep farm in Tamil Nadu (Easwaran, *et al.* 2009) [4], four sheep farm in Karnataka (Kumar *et al.* 2014) [7] and sheep in unorganized sector in Haryana (Priyanka 2019) [12]. In the present study, while two descript breeds and non descript breed showed resistance, Balangir breed showed susceptibility to fenbendazole anthelmintic. The higher efficacy of fenbendazole in the breed might be due to less number of treatments with the right dosage as well as good managerial practices.

The presence of anthelmintic resistance may be due to the selection of resistant genotypes within the parasitic population or reselection of resistant individuals already present in the population at a lower frequency. The use of anthelmintics increases the frequency of these resistant worms, so efforts should be aimed at reducing the further multiplication of these resistant worms.

Conclusion

The detection of anthelmintic resistance against fenbendazole in different sheep breed necessitates the urgency to acquire strategies that impede the development of anthelmintic resistance. There should be clear focus on the importance of correct dosage, drug rotation and proper management, as these practices are pivotal to delay the onset of benzimidazole resistance in different sheep breeds.

Acknowledgement

All the authors are thankful to All India Network Programme on Gastrointestinal Parasitism funded by ICAR and the Dean, College of Veterinary Science and Animal Husbandry, Bhubaneswar, for providing facilities to conduct the current research work.

References

1. Coles GC, Bauer C, Borgsteede FHM, Geerts S, Klei TR, Taylor MA *et al.* World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology* 1992;44:35-44.
2. Coles GC, Jackson F, Pomroy WE, Prichard RK, Von Samson- Himmelstjerna G, Silvestre A *et al.* The

- detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology* 2006;136:167-185.
3. Das M, Singh S. Anthelmintic Resistance to nematodes in sheep and goat farms in Hisar. *Journal of Veterinary Parasitology* 2005;19(2):103-106.
 4. Easwaran C, Harikrishnan JT, Raman M. Multiple anthelmintic resistance in gastrointestinal nematodes of sheep in Southern India. *Veterinarski Arhiv* 2009;79(6):611-620.
 5. Himmelstjerna Georg von Samson, Coles GC, Jackson F, Bauer C, Borgsteede F, CirakVeli Y *et al.* Standardization of the egg hatch test for the detection of benzimidazole resistance in parasitic nematodes. *Parasitol Research* 2009;105:825-834.
 6. Kumar S, Singh S. Detection of multiple anthelmintic resistances against gastrointestinal nematodes in sheep on central sheep breeding farm, Hisar. *Haryana Vet.* 2016;55(2):210-213.
 7. Kumar SC, Renukaprasad C, D'Souza Placid E, Pradeep BS. Evaluation of albendazole against sheep strongyles in farms of Karnataka state by different *in vitro* tests, *Anim. Sci. Res* 2014;43(5):330-339.
 8. Lata P. Studies on status of anthelmintic resistance in sheep and goat of Udaipur district (Rajasthan) A PG Thesis submitted to RAJUVAS, Udaipur 2018.
 9. Levecke B, De Wilde N, Vandenhoute E, Vercruysse J. Field validity and Feasibility of four techniques for the detection of *Trichuris* in simians: a model for monitoring drug efficacy in public health *PLoS Neglected Tropical Disease* 2009;3:366.
 10. Martin PJ, Anderson N, Jarett RG. Detecting Benzimidazole resistance with faecal eggcount reduction tests and *in vitro* assay. *Australian Veterinary Journal* 1989;66:236-240.
 11. Nanda S. Studies on Benzimidazole Anthelmintic Resistance in gastrointestinal nematodes of Goats in Dhenkanal District Odisha with special Reference to *Haemonchus contortus*, A PG thesis submitted to O.U.A.T BBSR 2017.
 12. Priyanka, Vohra S, Singh S, Sangwan AK. Cross Anthelmintic Resistance in *Haemonchus contortus* in Sheep of Unorganized Sector in Haryana, *Journal of Animal Research* 2019;9(5):256.
 13. Rialch A, Vatsya S, Kumar R. Benzimidazole Resistance in Gastrointestinal Nematodes of Small Ruminants of Uttarakhand. *Indian Journal of Animal Sciences* 2013;85(7):125.
 14. Sahu A. Molecular diagnosis of benzimidazole resistance in *Haemonchus* species of Ruminants; A PhD thesis submitted to O.U.A.T Bhubaneswar 2015.
 15. Sargison ND, Jackson F, Bartley DJ, Wilson DJ, Stenhouse LJ, Penny CD. Observations on the emergence of multiple anthelmintic resistance in sheep flocks in the south-east of Scotland, *Vet Parasitology* 2007;145:65-76.
 16. Singh R, Bal MS, Singla LD, Kaur P. Detection of anthelmintic resistance in sheep and goat against fenbendazole by faecal egg count reduction test. *J Parasit Dis* 2015;41(2):463-466.
 17. Soulsby E.J.L. *Helminths, Arthropods and Protozoa of Domesticated Animals*, 7th Ed. The English Language Book Society and Balliere Tindall, London 1982, 809.
 18. Swarnakar CP, Singh D. Indian perspective on anthelmintic resistance in gastrointestinal nematodes of small ruminants, In: *An update on diagnosis and control of parasitic diseases* Published by Department of Veterinary Parasitology, Veterinary College Shimoga, Karnataka 2017, 88-98.
 19. Taylor M, Coop R, Wall R. *Veterinary Parasitology* 3rd ed. Blackwell publishing, Oxford 2007.
 20. Waller PJ. The development of anthelmintic resistance in ruminant livestock. *Acta Tropica* 1994;56:233-243.
 21. Wolstenholme AJ, Fairweather I, Prichard R, von Samson-Himmelstjerna G, Sangster N. Drug resistance in veterinary helminthes, *Trends in Parasitology* 2004;20:469-476.